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The Changing Role of Animal Toxicology in Support of Regulatory Decisions

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The Clean Air Act is the basic U.S. Federal law for controlling air pollution. **Under Sections 108 and 109, primary (health) national ambient air quality standards (NAAQS) can be set for pollutants which are ubiquitous in the ambient air. The standard-setting process includes a comprehensive summary of scientific information on effects and controls in criteria and control techniques, and the selection of an appropriate standard which, in the judgment of the Administrator, protects the health of normal and susceptible subpopulations with an adequate margin of safety.**

Determining the adequacy of existing NAAQS or establishing new standards requires **that the scientific information base be evaluated to assess pollutant effects on public health. Improvements in this process can be accomplished not only through new health effects research, but also through improved use of currently available data. The commonality joining these two efforts is in the area of extrapolation modeling, which is the topic of this paper. Extrapolation modeling involves determining the effective dose delivered to the target organ of several species and the sensitivity of the target organ to that dose so that effective pollutant concentrations can be estimated across species. This in turn allows greater utilization of the results from animals in making judgments about the effects in man from exposure to a given pollutant.**

Integration of Human and Animal Data

Although, in the natural situation, man is exposed to a vast complex of interacting pollutants which may manifest themselves in acute or chronic disease, the protection afforded by NAAQS is presently based on single pollutants. It is immensely difficult for epidemiological studies quantitatively to identify chemicals which cause disease because of the many confounding variables. While controlled human studies offer the ability to relate cause and effect directly, in that exposure patterns can be accurately controlled and some confounding variables can be eliminated, they too have deficiencies. Because safety of the volunteer is of paramount importance, chronic exposures cannot be performed; only limited end points such as acute responses which can be measured with pulmonary function, clinical chemistry, or other noninvasive techniques, can be studied. This restricts the evaluation of pollutant effects to acute and typically reversible effects. Thus, there has been concern about the degree of adversity of such clinically observed effects and, therefore, their relevance to the setting of NAAQS. Of even greater concern is that with human studies, one can never investigate the full array of potential disease states induced by air pollutants.

Animal studies permit a complete evaluation of disease, in that the researcher has the choice of a wide range of concentrations, exposure regimens, chemical agents, biological parameters and animal species. Many physiological mechanisms are common to animals and man, so it can be hypothesized that if a pollutant causes a particular health effect in several animal species, it is likely to cause similar effects in man. However, quantitatively relating effective pollutant concentrations in animals to concentration responses in man is not currently possible.

Historically, animal toxicology has been used to illustrate the array of potential human effects

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and to elucidate mechanisms. However, the Clean Air Science Advisory Committee to EPA at a meeting February 5-6, 1981 in Washington, DC, recommended an increased direct utilization of animal data in reviewing and recommending primary NAAQS. For example, currently available quantitative chronic NO_2 exposure studies have used animals. However, the animal studies cannot be used directly to determine the need for an annual standard because of a lack of quantitative extrapolation models. Since animal studies clearly show that long-term exposure to NO_2 causes emphysema (*1-4*), the impetus is clear. If man is to be protected from such irreversible effects, additional efforts must be undertaken to develop extrapolation models useful in the setting of NAAQS. Pending availability of such models, animal and human data can be evaluated with a view towards increased understanding of potential effects on the public health.

A Case Study

The available evidence for considering the need for a short-term standard for NO_2 comes from epidemiological studies in homes using gas stoves compared to homes using electric stoves, from corroborative animal toxicological studies and from clinical studies on sensitive populations. Children in British homes with gas stoves experienced an increase in respiratory illness, prevalence of bronchitis and increases of "day or night cough" and "colds going to the chest" (*5, 6*). However, the NO_2 levels associated with these effects cannot be determined precisely since homes other than those of the study subjects were investigated (*7*). Thus, these studies can only provide qualitative support for similar investigations.

In a study of 6 US communities, children under age 2 living in homes with gas stoves had an increase in serious respiratory illness and a decrease in pulmonary function (*8*). This study and another related one (*9*) reported NO_2 levels found in homes with gas and electric stoves. Short-term peaks lasting minutes to hours in excess of 470 $\mu g/m^3$ (0.25 ppm) and even 940 $\mu g/m^3$ (0.5 ppm) were observed in the former. The 95th percentile of the 24 hr average NO_2 concentration was in the range of 38-113 $\mu g/m^3$ (0.02-0.06 ppm) for gas stove homes and 19-94 $\mu g/m^3$ (0.01-0.05 ppm) for electric stove homes.

Major uncertainties associated with these various epidemiologic studies were what specific agents(s) caused the response and what exposure level (concentration and averaging time) was associated with the reported effects. Animal studies, which were helpful in addressing these uncertainties, involved the use of an infectivity model in which animals are exposed to NO_2 or clean air and then exposed to an aerosol of viable microbes. Through bacterial defenses, the normal individual is quite capable of defense against the establishment of hazardous microbes. Any significant alteration in these pulmonary defenses would prolong the life of the infectious agents and permit their multiplication, which ultimately in the animal model results in death. If this animal model is to be used to reflect the toxicological response occurring in man, then the end point for comparison of such studies should not be mortality, since today few individuals die of bacterial pneumonias but rather should be the increased prevalence of respiratory illness. Such a comparison is proper, since both mortality (animals) and morbidity (man) are the consequences of a loss in pulmonary defenses. With the foregoing as background, the infectivity model data pertinent to the gas stove studies will be discussed.

In studies examining the interaction between concentration (C) and time (T) of exposure, although the $C \times T$ product remained constant, the response was significantly different when C and T were varied (*10*). The data indicated that concentration was more important than the length of exposure. Such data illustrate the need for obtaining and examining detailed monitoring data in epidemiological studies to determine the appropriate sampling time and frequency adequate to protect health. Thus, a simple arithmetic average could not predict the response.

Since many individuals, such as the subjects in the gas stove studies, are exposed intermittently rather than continuously, it is important to understand and compare these different exposure regimens. When comparing the response of animals to intermittent versus continuous exposure to a high and a low concentration of NO_2, it was found that at the lower concentration, the continuous exposure initially produced a greater response than the intermittent exposure, but after approximately 100 hr of intermittent exposure, the response was similar, regardless of the mode of exposure (*10, 11*). When the concentration was increased, the effect observed for the two exposure modes was never significantly different regardless of the time period used.

These studies were expanded (*12, 13*) to determine responses to single and multiple exposure spikes of various lengths as well as what effect these spikes have when superimposed on a lower continuous background concentration. The ratio of spike to basal concentration was 3:1 since this

mimics ratios frequently seen in urban communities. The greatest effect was seen when the short term spikes were superimposed on a low background level of NO_2. In this case, the exposed animals were not able to recover from the harmful effect of the spike, even though they spent a more extensive period of time in an atmosphere containing much less NO_2. However, when the animals were allowed to recover in clean air, there was recovery. These studies indicated the complexity involved in defining toxicological responses associated with various, but realistic, exposure patterns. They confirmed that NO_2 causes significant increases in mortality, i.e., loss of host defenses; however, the pattern and magnitude of the response were affected by the specific exposure regimen.

Although the gas stove studies alone provide useful information, the animal infectivity model data add support and elucidate significant uncertainties. The animal infectivity studies show that NO_2 was probably the causative agent in the epidemiological studies and that concentration of NO_2 is more important than length of exposure in increasing susceptibility to infectious pulmonary illness. The short-term peaks observed in the gas stove homes were likely the major causative factor in the effects observed. Examples can be described for other NAAQS in which the large animal toxicological data base provides evidence for potentially severe adverse effects in man. But without extrapolation models, this information can only be used qualitatively.

Developing Pulmonary Extrapolation Models

There are two major components to the pulmonary toxicological evaluation of the effects of NAAQS pollutants in man and animals. First, one must be able to estimate the dose to the specific target region of the lung as a function of exposure concentration (dosimetry). Second, for a particular biological end point (edema, morphological change, etc.), a damage function must be obtained which relates the effective delivered dose to the magnitude of the biological response observed (sensitivity). For example, if the same dose is delivered to a target site in a mouse and a person, is the response equivalent? Both components are vital for extrapolation of animal data to humans. And, of course, an adequate health effect data base must exist on which to make extrapolations.

Dosimetry

Extrapolation begins by estimating differences between man and experimental animals in the ratio of specific tissue dose to exposure concentration. To achieve these objectives, greater emphasis must be placed upon developing mathematical models for pollutant regional deposition in the lung. These models should incorporate species anatomical and ventilatory differences and physicochemical properties of the pollutant as parameters and should be based upon the factors which govern transport and removal of the pollutant.

Dosimetry for Particles. For particles, the overriding factors influencing regional respiratory tract deposition are those based upon aerodynamic properties, which in turn depend upon a variety of physical properties. The aerodynamic equivalent diameter D_{ae} is defined as the diameter of a unit density sphere which has the same settling speed (under gravity) as the particle in question of whatever shape and density (*14*). Aerosols of two entirely different chemicals, such as lead oxide and cadmium oxide, will deposit similarly in the respiratory tract if their aerodynamic characteristics (i.e., mass median aerodynamic diameter and geometric standard deviation) are the same.

When particles are inhaled, their aerodynamic properties, combined with various anatomical and breathing characteristics, determine their fractional deposition in the respiratory tract. With respect to respiratory tract deposition and clearance of inhaled aerosols, three regions can be considered: extrathoracic, the airways extending from the nares down to the epiglottis and larynx at the entrance to the trachea (the mouth is included in this region during mouth breathing); tracheobronchial region, the conducting airways of the lung from the trachea to the terminal bronchioles; pulmonary region, from the respiratory bronchioles to the alveoli (i.e., the gas-exchange region).

Historically, the most widely used regional deposition and clearance models were developed by the International Commission on Radiological Protection (ICRP) Task Group on Lung Dynamics (*15*) at a time when the available human data were primarily total deposition values for polydisperse and sometimes unstable aerosols. Although these models were not intended for broad application to environmental aerosols, they have been so applied by some scientists. Currently, available total and regional respiratory tract deposition in humans of monodisperse insoluble, stable aerosols of different sizes obtained under various

breathing conditions have been recently reviewed (*16*).

Nose breathing and mouth breathing provide contrasting deposition patterns for some respiratory tract regions. Particles of D_{ae} about 10 μm or larger are deposited in the extrathoracic region during nose breathing (*16*). This compares to about 65% deposition of particles of 10 μm D_{ae} under conditions of mouth breathing. On the other hand, extrathoracic deposition of particles smaller than about 1 μm D_{ae} is slight for both routes of breathing. The increased penetration of larger particles deeper into the respiratory tract with mouth breathing is reflected by experimental deposition data showing that tracheobronchial deposition of 8-10 μm D_{ae} particles is greater than 20%. About 10% of particles as large as 15 μm D_{ae} are predicted to enter the tracheobronchial region during mouth breathing (*17*).

Inhaled particles less than about 4 μm D_{ae} have pulmonary deposition fractions between 20 and 70%. For mouth breathing, as compared to nose breathing, the peak of the pulmonary deposition curve shifts upward from about 2.5 μm D_{ae} to 3.5 μm D_{ae} (*16*). The peak is much less pronounced for nose breathing (about 25% compared to about 50% for mouth breathing), with a nearly constant pulmonary deposition of about 20% for all sizes between 0.1 and 4 μm D_{ae}. Depending upon the tidal volume and breathing frequency, pulmonary deposition of particles 5 μm in aerodynamic diameter can vary from 5-50%. With mouth breathing, about 5-13% of particles 8-9 μm in aerodynamic diameter are deposited in the pulmonary region.

Regional deposition data of particles less than 3 μm D_{ae} in nose-breathing rats, hamsters, and dogs are available (*18, 19*). In these animal species, the relative regional distribution of particles less than 3 μm D_{ae} during nose breathing follows a pattern that is similar to regional deposition in man during nose breathing. Thus, in this instance, the use of rodents or dogs in toxicological research for extrapolation to humans entails differences in regional deposition of insoluble particles less than 3 μm D_{ae} that can be reconciled from available data. Before similar relationships can be determined for larger particles and other animal species, additional research must be conducted.

With regional deposition data (*16*) and information on tidal volume, breathing frequency, and lung surface area (*2*), an example can be given on how dosimetric comparisons can be made from an animal toxicological study in which rats are exposed to a 0.4 μm mass median aerodynamic diameter (MMAD) aerosol with a geometric standard deviation (σ_g) of 2.0. Regional deposition data allows one to compute how much of the aerosol mass is deposited. Then this estimate can be adjusted for differences in ventilatory parameters to obtain dosage comparisons between rats and man. If dose is expressed in terms of micrograms of the aerosol per square centimeter of lung per unit time, the pulmonary region of man receives approximately one-fourth of the dose of the pulmonary region of a rat, given that both breathe the same aerosol concentration. However, for the rat as compared to man, about a 10-fold greater dose is delivered to the tracheobronchial region. If sensitivity to a given dose were equivalent in man and rats, the toxicological results from the rat study would be quantitatively directly extrapolatable to man. The availability of the full range (0-15 μm D_{ae}) of particle size regional deposition data for man and several species of animals is the major limiting factor which prevents the widespread formulation of such dosimetric relationships.

Clearance of deposited particles also influences dosimetry. The chemical solubility of the gas or the particle is a prime determinant of the rate of clearance. The more rapid the clearance, the less time available for adverse effects at the site of original deposition.

Dosimetry for Gases. While aerodynamic properties provide a commonality for examining particulate deposition, gaseous deposition is more specific to the properties of the individual gas. The major factors affecting the uptake of gases in the respiratory tract are the morphology of the respiratory tract, the route of breathing, the depth and rate of breathing, physicochemical properties of the gas, the physical processes which govern gas transport, and the physicochemical properties of the aqueous material on the surface of the airways or gas exchange units. Discussions of general considerations useful in modeling respiratory tract transport and absorption of gases are available (*20-22*).

To illustrate how to compare toxicological data from experiments on guinea pigs and rabbits and to predict the exposure level equivalent in man, consider the following example. In man, rabbit and guinea pig, the pattern of O_3 deposition is similar, with one specific area of the lung (the respiratory bronchioles) receiving the maximal dose (*23*), a prediction correlating well with histopathological data (*24-26*). Suppose an experiment has been conducted in which guinea pigs were exposed to 2000 μg O_3/m^3 (1.02 ppm). Allowing for approximately 50% nasopharyngeal removal

(27), this exposure level corresponds to a tracheal O_3 concentration of 1000 μg/m³ (0.51 ppm). A 1000 μg/m³ (0.51 ppm) tracheal O_3 concentration in guinea pigs yields the same predicted respiratory bronchiolar dose as does a 500 μg/m³ (0.26 ppm) tracheal O_3 concentration in rabbits (23). This corresponds to a 420 μg/m³ (0.21 ppm) tracheal O_3 concentration in man. Thus, the model predicts that, as far as the O_3 dose to the respiratory bronchioles is concerned, comparable exposure levels occur if guinea pigs are exposed to 2000 μg/m³ (1.02 ppm) and rabbits to 1000 μg/m³ (0.51 ppm). If data on nasopharyngeal removal of O_3 in man were available, a comparable exposure level for man could be predicted.

Tissue Sensitivity

Even when the dosimetry is known, the biological response to a given dose may be different in various species. An accurate extrapolation must, therefore, take such potential differences into account. When accounting for species differences, the simplest possibility is when the mechanism of action is common among species, but input factors or repair factors affecting the magnitude of response have graded levels across species.

For a given pollutant, the ultimate mechanism of toxicity should be similar across species. For example, with O_3, the initiation of the reaction with unsaturated fatty acids involves a direct attack of O_3 on the carbon-carbon double bonds of the fatty acid (28). While these fatty acids and their double bonds are identical in all species, there are species differences in the proportion of classes of these unsaturated fatty acids. In addition, this initial O_3 reaction results in the formation of reactive intermediaries. These steps are influenced by such things as the concentrations of other chemicals (like levels of vitamin E or antioxidant enzymes) which also differ across species. In addition, repair mechanisms which have a great influence on the expression of toxicity differ across species. However, these factors ought not to override the basic similarities, so that it is feasible to develop pulmonary extrapolation models. For example, the fact remains that when monkeys, rats, cats, or mice are exposed to low levels of O_3, there is a sloughing of Type I alveolar lining cells and a replacement by Type II cells (29). For this type of effect, the sensitivity issue relates to the tissue dose-response characteristics and related mechanisms across species so that the human response can be predicted quantitatively.

Areas of Needed Research

Research is needed in dosimetry, sensitivity, and health effects to meet the ultimate goals of extrapolation. The more accurate the information on the various components comprising extrapolation models, the more quantitative the dosimetric estimates will be. Information on the biochemical composition, turnover and synthesis rates and regional thickness in the mucous and surfactant layers and the nature of the reactions of the pollutants with the components of these layers is needed.

Respiratory tract morphometric data are vital for dosimetry models of both gases and particles. The nature and structure of the respiratory tract in man and animals critically influences the deposition of both particles and gases since the relative contributions of the mechanisms affecting particle deposition and of gas transport processes vary as a result of this morphology. Morphometric data are available for some animal species (30-32), as well as the normal adult man (33-35), but need to be obtained for other animal species and for potentially susceptible human subpopulations, such as children, emphysemics, etc.

In estimating pollutant concentrations responsible for observed pulmonary effects, nasopharyngeal removal of the pollutant plays an important role. Experimental estimates of nasopharyngeal pollutant removal serve to determine appropriate starting concentrations when using convective-diffusion equations to model pollutant gas transport in the lower airways. Previously, upper airway studies on ozone (27, 36-38) sulfur dioxide (39-43) and nitrogen dioxide (40, 43) have been conducted. However, except for SO_2 (44-46) little is known about upper airway removal in man. Uptake during normal respiration versus exercise and for different breathing routes needs to be studied.

While the effective axial diffusivity in the lungs of man as a function of the mean axial gas velocity has been studied (4), this work needs to be extended to animals. The vertices imparted by the bifurcation angles and branching patterns in animal lungs may result in significantly altered diffusion coefficients in the conducting airways. This could change tracheobronchial dose curves, but it is not likely to influence pulmonary dose patterns, since molecular diffusion is the dominant mechanism facilitating gas exchange in the pulmonary region.

The thickness of the mucous layer in rabbits (47) has been used as the basis for assigning

thickness values in other species that have been studied thus far. Studies on mucous thickness in man and in other laboratory animals are not currently available and are needed before more definitive comparisons of conducting airway gaseous doses can be made.

The diffusion coefficient of various gaseous pollutants in mucus and surfactant needs to be established. Since the diffusion coefficient is important in establishing the mass flow rate of the gaseous pollutant from the lumen into the mucus and surfactant layer, better estimates of the diffusion coefficients would allow more accurate predictions of absolute differences in dose between species.

Regional deposition of coarse mode particles in normal adult man and in most animal species has not been thoroughly studied and significant improvement in knowledge of fine particle regional deposition in animals is also needed. To meet the mandate of the Clean Air Act to protect potentially susceptible subpopulations, deposition data for such populations are required. These populations would include children, individuals with chronic obstructive pulmonary disease, etc. Ostensibly, individuals may be at greater risk while exercising due to increased ventilation and consequently an increased dose and/or a different pattern of regional deposition. Some of these latter changes may reflect the switch from nasal to oronasal breathing with increased ventilatory demand. The influence of the varying degrees of oronasal breathing on deposition must be studied if the currently available data from experiments using mouthpiece breathing are to be fully utilized.

In determining species susceptibility, it is necessary to deliver a known dose to the cells/tissue of several species and measure a variety of biological responses. Major categories could include host defense mechanisms against infectious disease, pulmonary physiology, lung biochemistry/pharmacology, and pulmonary morphology. In these investigations, the dose would be delivered in two ways: *in vitro* so the actual dose is known, and *in vivo* (inhalation) in cases where dosimetry estimates are available and where the parameter is not ammenable to an *in vitro* approach (i.e., pulmonary physiology).

When extrapolation models are available, they must be applied to the existing data base. The currently available data base consists of mostly scientifically sound studies conducted in various laboratories with different techniques. The problem can be visualized by examining summary tables in the O_3 Criteria Document (*29*). There are almost no series of studies which permit sensitivity comparisons since several different species were used. There was no commonality of sex and age of the animal, concentration and length of pollutant exposure, or measurement techniques. The entire data base for NAAQS pollutants must be critically reviewed for gaps relevant to extrapolation models. For each pollutant, such a search might reveal various health end points for which increased knowledge would directly aid in extrapolation.

Although the emphasis of the above discussion has been on quantitative extrapolation, qualitative aspects cannot be ignored which relate to improving knowledge of the mechanisms of toxicity for the most significant health effects. Much information is available on these mechanisms, so again the issue is refinement of the data base by highly goal-oriented studies. Knowledge of mechanisms is the cement that binds together the various elements of quantitative extrapolation.

Summary

Environmental toxicologists are confronted with the formidable task of interpreting the results from epidemiological, human clinical and animal studies on air pollutants and assessing their relevance and implications concerning pollutant levels to which man can be safely exposed. Usually, the fraction of the total available toxicological data base represented by epidemiological and human clinical studies is quite small compared to the data available from animal studies. This data imbalance stems from the nature and inherent limitations of epidemiological and human clinical studies. By contrast, animal experimentation provides the choice of a wide range of concentrations, exposure regimens, chemical agents, biological parameters and animal species. Relating dose-response results obtained in animal studies to the human experience is currently difficult.

Many of the above considerations can be resolved through the development and use of pulmonary extrapolation models which account for species differences in dosimetry and tissue sensitivity to the pollutant and the selective improvement of the health effects data base. This paper has focused on factors which must be considered when developing such models. Animal studies are the only avenue available for investigation of the full array of potential disease states induced by air pollutants. However, without the development and use of pulmonary extrapolation models, the full impact of air pollutants on the public health cannot be quantitatively and accurately assessed.

This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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